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8/28/20

Made new 1X MBSH and 1X DS. Put away dishes. Autoclaved 1L bottle. Other housekeeping tasks. -EF

8/30/20

Made new stock and 1x DS and MBSH. -EF

9/7/20

Continued working on the cell culture project section of the lab wiki. Also finished my introductory presentation regarding the cell culture project to share with interested members of the lab. - BS

9/8/20

Finished Covid-19 Modeling training task. -AR

9/14/20

Prepared for my zoom meeting. Attempted to give the meeting, but the students who said they would show up did not. - BS

Performed two electrophysiology experiments, SCN Block with MTSEA modification (efb091420) and Anion substitution w/o modification (ef091420). Did dishes. Autoclaved bottle. -EF

9/18/20

Injected oocytes with .02% L102C, .5% BAR. -EF

9/20/20

Prepared for lab meeting. Posted last week's and this week's lab meeting recording. Started preparing for upcoming lab meeting. -EF

9/21/20

Performed electrophys expt (ef092120) and analyzed; after this and last week's expts I concluded there is something wrong with 90% NaSCN, so I made new 90%NaSCN. Did dishes. -EF

9/23/2020

Prepared a presentation on cell culture for the students who are interested. I plan to have another small zoom call next week. - BS

9/25/2020

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Autoclaved 3L DI water; made 1 L LB broth. Injected oocytes with .02% L102C, .5% BAR. Discarded old oocytes -EF

Made some 70% ethanol. Finished working on my cell culture presentation for next week. Filmed videos to send to the students interest in cell culture that should help them get more oriented to the layout of the lab. I noticed the CO2 tank for our incubator is running very low. Due to this, I'm waiting to turn on the cell incubator and being cell culture until Monday - BS

9/28/20

Turned on the cell incubator and made sure it was both set to the desired settings for cell culture, as well as was functioning properly. It appeared to be working fine and hadn't lost water (which it has done multiple times in the past). I disinfected and disposed of the old cell lines left in the incubator, and autoclaved the old media. I disinfected the interior of the cell incubator and replaced the water in the humidity pan. Went to the purchasing department to look for a replacement CO2 container, and they had multiple we could use. Terry (purchasing) said we could contact facilities to try and bring the tank up to the fifth floor if the elevator stays broken for a while. Also completed required ATSU training - BS

Did electrophys experiment (efb092920). Lots of trouble with glass pipettes, I think they may be too narrow at the tips. I checked them under the microscope and the openings are wide enough. Autoclaved bottles. Sorted through expts that need to be done. -EF

9/29/20

Mutated sACE2 to evaluate for efficacy, and reported the DOPE score to Alec. -AR

9/30/20

Thawed one tube of frozen LM45 cells. Our CO2 is depleting faster than I'd thought, and we will probably need a new CO2 container by the end of next week. Finished preparing for my cell culture zoom call. - BS

10/2/20

Injected oocytes with .02%L102C, .5% BAR. -EF

Gave a zoom call regarding cell culture techniques to the students who were interested. Went in to lab to check the health of LM45 cells I thawed on wednesday. Of the three dishes I that should contain LM45 cells, one dish contained cells, while the other two dishes appeared to only contain cell fragments/dead cells. I'll attempt to passage the cells on Monday and reassess their health. The cell fragments/dead cells appeared to be there immediately after plating the cells from thawing, so I believe the cells either died while being frozen/freezing for the past 6 months, or died during the thawing process, but before being plated. I'll try and see if I can troubleshoot next week. - BS

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10/5/2020

I checked the viability of the cells thawed last week. Two of the dishes appear to have cells in them, but they are not confluent enough to passage or refreeze yet. Emma helped me record a video outlining sterile technique and setting up the tissue culture hood prior to performing cell culture techniques. I imported the videos and uploaded the unedited clips to the google drive. I'll post on slack the final video is edited and finished. - BS

Sorted oocytes. Recorded blake explaining sterile technique. Started electrophys expt, but pipettes became blocked. Oocytes seem healthy, so I determined it was the pipettes that are the issue. Worked to try and fix their shape. Scheduled frog surgery. Replaced injection pipette. Ordered NaI anhydrous. -EF

10/7/2020

Talked with purchasing and facilities about ordering a new CO2 tank. Facilities will be up either Thursday or Friday to bring the tank. I talked with Dr. Baer about how to connect the new tank, and I should be able to connect it on Friday (although I might have Dr. Baer check to see if it's hooked up correctly. I planned on filming a video on how to view cells under light microscopy today, but the CO2 tank is completely empty and I'm afraid opening the incubator will drop the CO2 levels and kill the cells we are incubating before Friday, so I'll film it next week. I plan to passage the thawed cells on Friday. I added some more information to the "Cell Incubator Operation" and "Literature Review" pages on the lab wiki. I found a paper on designing pegRNA and shared it on the google drive (CRISPR Project → Literature for Referencer or Investigation) and I read part of it and glanced at the pegfinder tool it mentions. - BS

Attempted electrophys expt but pipettes are not working. Tried fixing them by changing parameters on pipettes puller but both stopped working. -EF

10/9/2020

I attempted to passage one of the dishes of thawed cells, but they didn't look promising. I'll assess their health next week, but I'll probably end up trying to thaw another tube next week. We received the new CO2 tank, and its in room 515 next to the incubator. However, I couldn't change the tank today because I'll need a second pair of hands to help me put in the chained region before replacing the tubes. I think we'll have enough CO2 until Monday. - BS

Injected oocytes with .02% L102C. .5% BAR. -EF

10/12/20

Emma helped me move the new CO2 tank within the chained region by the incubator, and I moved the old tank out of the chained region. I called facilities to help me make sure the CO2 tank was changed properly, and it was changed. Gabby finished editing the sterile technique

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Emma helped me film last week, and I put it on the google drive (CRISPR Project → Videos) and on the lab wiki (in the sterile technique page). I disposed of the old cell dishes from trying to thaw cells last week. I read more about how to properly thaw out cells, and I plan to try that again on Wednesday. - BS

Performed electrophysiology experiment (ef101220), anion sub on .02%L102C .5%BAR. Still needs to be analyzed. Filtered IBMX. Took and uploaded pictures of oocytes. Pulled pipettes. Tried transferring DI water, during it the tank fell and broke. Looking into autoclaving the extra FR tank to use instead. Made new AB-AF MBSH.

10/14/2020

I disposed of the last dish from the last thawing attempt; there were no viable cells. I think the cells in the last round of thawing either died while freezing, or died/burst during the thawing process. I changed the protocol this time to include transportin the cells from the -80C freezer to our lab in an ice bath containing the frozen gel packs from Freezer B. If we had dry ice, that would be preferred. Once in the lab, I placed the tube of cells into a pre warmed water bath at 37C until most of the cells were visibility thawed. From here, I moved the cells to the tissue culture hood and combined the cells with 7.5 mL of pre warmed (~30-37C) growth media. I mixed the cell/media solution and then plated in between 3 plates. After checking the viability, two of the three dishes appeared to have cells in them. - BS

10/16/2020

Checked the cells I thawed on Friday. 2 of the 3 dishes (dishes #1 and #2) had healthy looking cells, so I left them to grow. Dish #3 also had cells, but I was worried it was infected, so I disinfected the dish with bleach and disposed of it. I updated the “thawing cells” protocol on the google drive to reflect the changes I made during this round of thawing cells. In the future it may be worthwhile to look into using liquid nitrogen to freeze cells, as cryostor media is not recommended for storage of longer than a few months of cells (per manufacturer). Got Lucas’s mail with Emma and put the primer in Freezer A and the chloroform in Fridge A.The new water jug is on the desk by electrophysiology station #2. - BS

Got mail with Blake. Will autoclave new water jug monday. Performed electrophysiology experiment (efb101220), .02%L102C, .5%B2AR. Pipettes are fine and so are the NaI and NaSCN solutions, but now conductance is going up with NaBr and $NaNO_3$ solutions, so I will remake those monday and see what happens. Injected oocytes with .02%L102C, .5%BAR. -EF

10/19/20

Performed electrophysiology experiment (ef101920), .02% L102C, .5%BAR with NEM modification. Replaced tubing and cleaned oocyte holder, hoping that makes experiments go more smoothly. Got more DI water. Will wait to see how experiment goes with new tubing before

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making new solutions. Talked to Dr. N and he agrees this is a good course of action. Uploaded oocyte pictures. Scheduled frog surgery. -EF

10/21/2020

Removed the old alcohol from the Mr. Frosty, cleaned it out, and left it to dry. Passaged the two dishes of LM45 cells into 3 dishes total. I plan to freeze 2 dishes next week, and record a video on how to freeze cells. Read more about the paper on the google drive detailing using prime editing to edit F508del in vitro. Looked up sequence of WT CFTR and a sequence for G551D CFTR and started looking into designing pegRNA for G551D using pegfinder. - BS

Injected .02% L102C, .5% BAR. Autoclaved flask (Ellen). Performed an analyzed ef102320, .02%L102C, .5%BAR; seems to have gone much better, but need to do secondary analysis, waiting on relative permeability sheet. Did some secondary analysis. Pulled new pipettes. Did dishes. -EF

10/26/20

Performed and analyzed experiment, ef102620, .02% L102C, .5% BAR SCN block with NEM modification. Filmed blake freezing cell protocol. Checked pipettes. Recorded oocyte health. Started learning secondary analysis. -EF

Froze two tubes of LM45 cells in the Mr Frosty/-80 C Freezer. Will remove them from the Mr Frosty on Wednesday, unless Bonnie is able to on Tuesday. Emma helped me record the process, and I'll go through the clips and send them to Gabby later this week. - BS

10/30/20

Injected oocytes, .02% L102C, .5% BAR. Started electrophysiology experiment (ef103020), but no activation. Gathered list of publishable experiments. Made new MBSH with AB and AF. Autoclaved Ellen and old MBSH with AB and AF. Performed electrophysiology expt efb103020, .02%L102C, .5%BAR, SCN block w/ NEM mod., still needs to be analyzed. -EF

11/2/2020

Pre-plated 4 wells of LM45 cells for transfection tomorrow with eGFP and eGFP+Cas9 tomorrow. I plated at a density of 125,000 cells per well. I checked/updated the protocol I made last semester for pre-plating, and I updated it on the lab wiki and google drive. I'll likely record a video on pre-plating and transfecting next week. - BS

11/3/20

Started 2 experiments, but no expression; oocytes seemed dead. Pulled electrophys pipettes. Pulled injection pipettes. Continued working on list of publishable experiments. Got more DI water for above the sink. Analyzed efb103020. -EF

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Planned to transfect eGFP and eGFP + Cas9 today, but the cells were not confluent enough to be transfected. Will try check the cells and try the transfection tomorrow. - BS

11/4/2020

Transfected eGFP and eGFP+Cas9. Will observe on Friday/Monday. Read more about the prime editing system to discuss over zoom on Friday. - BS

11/6/20

Replaced injection pipette. Injected oocytes with .02%L102C, .5%BAR. Oocytes don't look too healthy, so I am skeptical about how experiments will go next week. -EF

Met with Gabe, Meghan, and Kylie via zoom to discuss prime editing. Checked cells that were transfected on Wednesday. The dishes transfected with eGFP were fluorescing, but the cells transfected with eGFP + Cas9 were not. Will check again on Monday - BS

11/9/2020

Observed cells under fluorescence again. eGFP+Cas9 did not fluoresce. I will look into investigating why transfecting eGFP+Cas9 has not been successful. We'll probably need to order serum free media soon, as it might help transfection efficiency. Passaged LM45 cells. Recorded a video on how to operate the microscope in 504/observe cells under fluorescence.- BS

11/10/20

Started electrophys expt but oocytes not healthy enough. Put away dishes. Filtered I+I waste. -EF

11/11/2020

Communicated parts of the upcoming presentation with Kylie, Meghan, and Gabe. Uploaded the unedited clips of me explaining how to observe cells under fluorescence. Looked at the plasmid images of eGFP + Cas9 again to ensure eGFP was present. Did some editing on the lab wiki - BS

11/16/20

Got rid of old oocytes. Autoclaved solutions to be discarded. Recorded Blake. Continued organizing experiment analysis and quality. Made new MBSH AB+AF. -EF

Plated LM45 cells for transfection of eGFP and Cas9. Emma helped me record the video clips. - BS

11/20/20

Continued sorting and searching through data. Started secondary analysis. -EF

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Transfection of eGFP was successful, and transfection of Cas9 was assumed to be successful. Did housekeeping/lab cleanup. I looked at the sequencing results for the DNA labeled Cas9 + eGFP and concluded that the plasmid is actually pCas0+eGFP, not pCMV-BE2. This means all of the attempted transfections with this DNA have failed. - BS

11/23/2020

Passaged LM45 Cells. Uploaded 3 sets of video clips and told Gabby about them for editing in the future. Tried to upload all of the CRISPR Protocol videos to the new lab wiki but will have to spend time figuring out how to do that in the new format over break - BS

11/24/20

Autoclaved flask. Copied data to work on from home. -EF

12/2/20

Made new 1X MBSH with AB +AF. -EF Injected oocytes: 50 ppm. .5% BAR and .02%L102C, .5% BAR. Posted protocol for making 1X MBSH with AB +AF. -EF

12/3/2020

Tried searching for pCMV-BE2 (prime editing) DNA, but do not know where it is. We ordered it before I was in charge of the CRISPR project, and I have not been told where the DNA is. I checked the sequencing results from February for our DNA labeled “Cas9 + eGFP” again, and I am more confident the DNA does contain eGFP, therefore is not the pCMV-BE2 DNA. This means we need to find the pCMV-BE2 DNA, and figure out why the Cas9+eGFP transfetions don’t work. - BS

12/4/20

Called ITS and installed sigma plot on computer A. Sorted oocytes. Performed secondary analyses using sigma plot; can be found on G drive. Made new 1X DS w/ Hepes H and Hepes Na. -EF

12/6/20

Continued secondary analysis of NaSCN blocks using sigma plot. Uploaded the files to google drive. -EF

12/7/20

Performed electrophysiology experiments on 50ppm R104C; ef120720 (SCN block with ES mod) efb120720 failed; efc120720 failed. Analyzed ef120720. Made new 90% NaI, 90% NaBr, 90%NaSCN, and 90%NaNO3. Autoclaved bottles. Got DI water. -EF

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12/8/2020

Passaged LM45 cells. Arranged with Bonnie for her to passage the LM45 cells each week over break. Bonnie told me Dr. Baer no longer has thawed LM45 cells, so we cannot use his lab to reestablish our line if we needed to right now. We have two tubes of LM45 and 2-3 tubes of Lm45-CD47 frozen right now though. Found pCMV-BE2 DNA in Box17 in Freezer C, but the concentration is not labeled.- BS

12/9/20

Sorted oocytes.Performed electrophysiology experiments: ef120920 (50ppm R104C Anion Sub with ES mod) and analyzed, efb120920 (50ppm R104C Anion Sub ES mod) terminated, efc120920 (50ppm R104C Anion Sub with ES mod). All experiments from this week are still on the station 1 computer, need to be transferred to the G drive. Pulled pipettes. Made new 1X MBSH (in fridge A). Made liberase aliquots (in -80 freezer) -EF

12/10/20

Made new FR. -EF

12/11/20

Checked oocytes. Measured osmolarity of new FR, 1X MBSH and 1X DS. Measured pH of new FR. Transferred electrophys data from this week. -EF